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TECHNICAL MANUSCRIPT 269

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UNITED STATES ARMY
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Project 1C014501B71A

April 1966

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Macaca mulatta were found to be susceptible to infections with group C arboviruses following subcutaneous inoculation. Infections engendered by Oriboca, Marituba, Murutucu, and Apeu viruses were consistent in time of onset, duration, and level of viremia. The appearance of viremias with Itaqui virus was occasionally inconsistent and with Caraparu virus the appearance of viremias was very irregular. Overt signs of systemic illness in monkeys infected with any of the group C viruses used were limited to fevers, which were detected in only a few animals. Infections with these viruses appeared to be noncontagious for uninoculated monkeys that were exposed by limited contact to infected monkeys in the viremic phase.

Cross-immunity was demonstrated by monkeys that recovered from one virus infection and were challenged with related heterotypic viruses.

I. INTRODUCTION

Several group C arboviruses are etiologic agents of febrile illnesses in humans and have been isolated by Causey et al.[1] and Shope et al.[2] from sentinel animals and mosquitoes in the rain forests of Para, Brazil. A species of monkeys native to South America, Cebus apella, was selected as sentinel host because of its availability and its susceptibility to viral infections, and because it could be obtained from areas where these group C viruses are neither endemic nor enzootic. The rhesus monkey, Macaca mulatta, is native to areas where no group C arboviruses have been identified, and is more readily available in the United States for experimental purposes. For these reasons it was desirable to investigate the susceptibility of this host to group C viruses.

II. MATERIALS AND METHODS

A. VIRUSES

Prototype strains of Oriboca, Murutucu, Marituba, Caraparu, and Apeu viruses were used in these studies. Strain AN 12752 of Itaqui virus was used because the prototype strain AN 12797 was not available. Seeds were prepared from infected suckling mouse brains as 10% suspensions in borate-buffered saline that contained 20% calf serum. Virus strain numbers, passage histories, and titers in suckling and young adult mice, expressed in $\log_{10}$ median lethal doses (LD_{50})[3] are presented in Table 1.

B. MONKEYS

Monkeys, Macaca mulatta, weighing between 5 and 8 pounds, were bled for pre-inoculation serum samples as soon as they were received in the laboratory. They were seated in primate chairs and rectal thermocouples were attached to a device for automatic recording of temperatures at 40-minute intervals. The monkeys were allowed 2 days to adapt to their new environment before being inoculated with virus. Only one viral agent was studied at a time to avoid accidental exposure of the monkeys to heterotypic agents.

C. ASSAY FOR VIREMIA

A blood sample was obtained daily from each monkey for 7 consecutive days after inoculation of virus. Each 1.0-ml sample of blood was

immediately diluted 1:10 in beef heart infusion broth and 0.03 ml of this dilution was inoculated into 10- to 14-gram Swiss mice by the intracerebral (ic) route to detect the presence of infectious virus. The remainder of each diluted blood sample was frozen and stored at -60 C until assayed for virus titer. Because Apeu virus was not consistently lethal for 10- to 14-gram mice, suckling mice were employed as the test animals for this agent.

TABLE 1. TITERS OF GROUP C VIRUS SEEDS IN MICE

Virus	Strain	mouse Passages	Log_{10} LD_{50} per ml of Seed[a] Suckling Mice IC[b]	Young Adult Mice IC	Young Adult Mice IP[c]
Oriboca	AN 17	13	6.8	7.0	6.5
Itaqui	AN 12752	8	7.8	7.6	6.0
Murutucu	AN 974	11	7.6	7.3	6.5
Marituba	AN 15	5+	6.3	6.0	5.0
Caraparu	AN 3994	12	5.8	5.9	3.6
Apeu	AN 848	7	6.5	1.0	1.0

a. 10% infected suckling mouse brain.
b. Titration by intracerebral route.
c. Titration by intraperitoneal route.

D. NEUTRALIZATION TESTS

Serum samples were obtained from each monkey approximately 10 and 21 days after inoculation of virus. These samples were compared with pre-inoculation serum samples for neutralization titers by the constant-serum varying-virus technique. Serum-virus mixtures were incubated 30 minutes at 37 C and 2 hours at 4 C, then were inoculated into mice by the intraperitoneal route, a method more sensitive in detecting neutralizing antibodies than the intracerebral route. In assays for Apeu virus antibodies, 1- to 4-day-old suckling mice were used; 10- to 14-gram mice were used in assays for antibodies against the other viruses.

III. EXPERIMENTAL RESULTS

Monkeys that were inoculated by the subcutaneous route with Oriboca, Murutucu, Marituba, or Apeu viruses developed infections as indicated by the data presented in Table 2. Because quantitative viremic responses were uniform among animals that received similar inocula, the titers were averaged.

Animals that were administered 1 x 10^6 mouse intracerebral LD_{50} ($MICLD_{50}$) of Oriboca virus had detectable viremias within 24 hours; no viremias were observed until the second day after inoculation in animals that received 1 x 10^3 $MICLD_{50}$. Viremias reached a peak titer of approximately 10^6 per ml of blood on the second or third postinoculation day. By the fifth or sixth day the viremias subsided completely.

TABLE 2. VIREMIA TITERS OF INFECTED RHESUS MONKEYS

Virus Inoculated	Dose, $MICLD_{50}$	Number of Monkeys	Log Mean Virus Titer per ml of Blood on Postinoculation Day						
			1	2	3	4	5	6	7
Oriboca	1 x 10^6	4	3.2	5.9	5.1	3.1	0[a]	0	0
	1 x 10^3	4	0	5.4	6.0	3.6	Tr[b]	0	0
Murutucu	2 x 10^6	5	2.9	5.0	5.2	3.7	Tr	0	0
	2 x 10^3	3	0	4.7	6.0	5.4	3.2	0	0
Marituba	6 x 10^5	3	3.4	5.0	5.5	Tr	0	0	0
	6 x 10^3	3	3.0	5.6	6.1	3.0	Tr	0	0
Apeu	5 x 10^6	3	3.3	4.3	5.1	3.2	0	0	0
	5 x 10^3	3	Tr	4.3	6.0	3.3	Tr	0	0

a. 0 = no virus detectable.
b. Tr = trace amounts of virus detected but titer was not estimable.

In monkeys inoculated with Murutucu, Marituba, or Apeu viruses, viremic patterns were similar to those observed with Oriboca virus infections. Circulating virus was detected through the first 5 days of infection with maximum titers occuring on the second or third day. A 1000-fold reduction in the inoculated dose of Murutucu or Apeu virus resulted in a one-day delay in the appearance of a viremia but did not alter its duration. A 100-fold reduction in the inoculated dose of Marituba virus resulted in no appreciable delay in the initiation of the viremia; however, the viremia persisted 1 day longer in monkeys administered the smaller dose of virus.

The viremic patterns of monkeys inoculated with Caraparu virus were markedly different from those observed with infections of the viruses described above. As seen from the data in Table 3, there was no uniformity in the time of appearance of viremias. When viremias appeared they persisted no longer than 2 days and usually were of very low titer. One of the 6 monkeys inoculated (M 67) failed to develop any detectable viremia during the 7-day test period.

Monkeys that were experimentally infected with Itaqui virus also demonstrated some variation in viremic patterns. However, viremias were generally more consistent in appearance and duration than those with Caraparu virus infections. The data in Table 4 reveal that in all but one monkey (B 7), measurable titers of Itaqui virus were circulated the first 3 or 4 days after inoculation and frequently persisted in trace amounts for at least 7 days. No significant differences in viremic levels were noted between monkeys inoculated with different doses.

TABLE 3. VIREMIA TITERS OF RHESUS MONKEYS INOCULATED WITH CARAPARU VIRUS

Monkey Number	Virus dose, $MICLD_{50}$	Log_{10} Virus Titer per ml Blood on Postinoculation Day						
		1	2	3	4	5	6	7
L 99	6×10^5	0[a]	0	Tr[b]	0	0	0	0
M 67	6×10^5	0	0	0	0	0	0	0
D 28	6×10^5	Tr	0	3.9	Tr	0	0	0
M 69	6×10^3	0	4.1	3.3	0	0	0	0
M 58	6×10^3	0	0	0	0	0	2.7	2.7
M 34	6×10^3	0	0	2.9	0	0	0	0

a. 0 = no virus detectable.

b. Tr = trace amounts of virus detected but titer was not estimable.

TABLE 4. VIREMIA TITERS OF RHESUS MONKEYS INOCULATED WITH ITAQUI VIRUS

Monkey Number	Virus dose, $MICLD_{50}$	Log_{10} Virus Titer per ml Blood on Postinoculation Day						
		1	2	3	4	5	6	7
B 7	1×10^6	Tr[b]	0[a]	0	0	0	Tr	0
D 18	1×10^6	3.9	2.9	2.8	Tr	Tr	Tr	0
Y 71	1×10^6	3.7	2.6	2.5	0	0	0	0
N 1	1×10^6	4.1	3.1	2.5	0	Tr	0	Tr
5A93	1×10^6	2.5	3.0	Tr	Tr	0	0	Tr
Y 50	1×10^3	Tr	4.9	4.0	4.0	Tr	Tr	0
3A29	1×10^3	0	0	0	4.5	0	0	0
Y 92	1×10^3	3.0	2.5	Tr	2.5	Tr	Tr	Tr
Y 84	1×10^3	3.0	3.2	2.5	Tr	Tr	Tr	Tr

a. 0 = no virus detectable.
b. Tr = trace amounts of virus detected but titer was not estimable.

All monkeys that developed detectable viremias after inoculation with group C viruses elicited significant levels of neutralizing antibodies against the respective infectious agents. Monkey M 67, which failed to develop a viremia after inoculation with Caraparu virus, was the only animal that had pre-existing neutralizing antibodies. No increase of neutralization titer occurred in the serum of this animal 20 days after inoculation.

In some monkeys infected with Oriboca, Apeu, Itaqui, or Murutucu viruses the only signs of illness observed were occasional, sporadic, low-grade fevers. These fevers were usually limited to elevations of nocturnal temperatures occurring between the second and seventh postinoculation day. Nearly all of the fevers were of such low magnitude that they probably would not have been apparent if an automatic recording apparatus had not been used. None of the monkeys inoculated with Marituba or Caraparu viruses developed discernible fevers or other signs of illness.

A. CONTACT TRANSMISSION EXPERIMENTS

One or two uninoculated monkeys were seated in close proximity and were limited in contact with each group of experimentally infected monkeys to determine whether these viruses could be transmitted from animal to animal by contact. None of the uninoculated animals developed detectable viremia, fever, or antibodies.

B. CROSS-PROTECTION EXPERIMENTS

Because of serological relationships among these viruses reported by Casals and Whitman[6] and by Shope and Causey,[5] it was of interest to determine whether monkeys that recovered from infection with one group C virus would be refractory to infection with another. The results of several such cross-challenges are summarized in Table 5. Monkeys that recovered from infection with Oriboca, Marituba, Murutucu, or Caraparu viruses possessed marked resistance to infection with related, heterotypic viruses. Resistance was demonstrable by the lack of, or very low levels of, postchallenge viremia. Generally, a monkey's prechallenge serum was able to neutralize completely the challenge virus. In two cases where neutralization was not complete in the prechallenge serum, increases in neutralization indices were observed in sera taken 20 days after challenge.

TABLE 5. RESULTS OF CHALLENGE OF RHESUS MONKEYS WITH GROUP C ARBOVIRUSES AFTER RECOVERY FROM INFECTION WITH RELATED VIRUSES

Challenge		Previous	Number of	Characteristics of
Virus	Dose[a]	Infection	Monkeys	Postchallenge Viremias
Murutucu	2×10^6	Oriboca	2	Not detectable
Caraparu	6×10^5	Marituba	2	Not detectable
Caraparu	6×10^5	Murutucu	1	Not detectable
Caraparu	6×10^5	Murutucu	1	Trace on 6th day only
Apeu	5×10^6	Oriboca	1	Not detectable
Apeu	5×10^6	Caraparu	1	Not detectable
Marituba	6×10^5	Oriboca	1	Trace on 1st day only
Marituba	6×10^5	Murutucu	1	Not detectable
Marituba	6×10^5	Murutucu	1	Trace on 6th day only

a. $MICLD_{50}$ inoculated by the subcutaneous route.

IV. DISCUSSION

The evidence of viremia and specific antibody response that was demonstrated in these experiments shows that the simian species, Macaca mulatta is susceptible to infection with group C arboviruses. With Oriboca, Murutucu, Marituba, and Apeu viruses the induced viremias were of sufficient uniformity in onset, duration, and titer to qualify this species for experimental studies with these agents.

The observed resistances of some rhesus monkeys to Caraparu and Itaqui viruses could possibly be accounted for by (i) the lack of virulence of these viruses for this host, or (ii) by an acquired immunity that the monkeys developed in their natural habitat by an experience with a related virus. Serological evidence supports the hypothesis that group C arboviruses are enzootic in rhesus monkeys indigenous to India. Scherer and Miura[6] reported the presence of Oriboca virus neutralizing antibodies in one of three Indian rhesus monkeys tested, and we found Caraparu virus neutralizing antibodies in one of six monkeys tested. In another study, Borman* found pre-existing antibodies that neutralized Oriboca virus in two of 12 rhesus monkeys tested. In view of the potential pathogenicity of these viruses for humans a concerted effort may be warranted to find and identify group C viruses in areas of India inhabited by rhesus monkeys.

Although these viruses were infectious for rhesus monkeys, the diseases appeared extremely mild in this host and in many respects were comparable to infections described by Causey et al.[1] and Shope et al.[2] in Cebus monkeys. In infections initiated by subcutaneous injection of virus neurological signs or other overt signs of illness were not evident. Minor deviations from normal diurnal temperatures occurred in some animals.

It was possible to demonstrate immunologic overlap in rhesus monkeys by heterotypic challenges. These results were compatible with those reported by Casals.[5] Recovery from infection with one group C virus induced immunity to selected viruses of the same group. In most of the monkeys this immunity was demonstrable by a complete or nearly complete suppression of viremia by the challenge virus.

The presence of cross-neutralizing antibodies detected in the sera of immune monkeys prior to challenge probably accounted for this protective overlap. These results emphasize the close antigenic relationships that exist among these viruses.

The failure of uninoculated monkeys to contract infections when placed in close proximity and limited contact with viremic monkeys implied either that the viruses were not shed in the excretions of the infected animals or that rhesus monkeys are relatively insusceptible to infection by the respiratory or oral routes.

* Borman, E.R. 1966. Unpublished results.

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